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Original article

Prognostic value of insulin-like growth factor 1 and insulin-like growth factor binding protein 3 blood levels in breast cancer

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ABSTRACT

High circulating insulin-like growth factor 1 (IGF-1) levels are firmly established as a risk factor for developing breast cancer, especially estrogen positive tumors. The effect of circulating IGF-1 on prognosis once a tumor is established is unknown. The authors explored the effect of IGF-1 blood levels and of it's main binding protein, IGFBP-3, on overall survival and occurrence of second primary breast tumors in breast cancer patients, as well as reproductive and lifestyle factors that could modify this risk. Patients were accrued from six hospitals in the Netherlands between 1998 and 2003. Total IGF-1 and IGFBP-3 were measured in 582 plasma samples.

No significant association between IGF-1 and IGFBP-3 plasma levels and overall survival was found. However, in a multivariate Cox regression model including standard prognostic variables high IGF-1 levels were related to worse overall survival in patients receiving endocrine therapy (HR = 1.37, 95% CI: 1.11, 1.69, *P* 0.004). These data at least indicate that higher IGF-1 levels, and as a consequence most likely IGF-1-induced signaling, are related to a less favorable overall survival in breast cancer patients treated with endocrine therapy. Interventions aimed at reducing circulating levels of IGF-1 in hormone receptor positive breast cancer may improve survival.

Interaction of insulin-like growth factor 1 (IGF-1) with its key receptor, the IGF-1 Receptor (IGF-1R), gives strong proliferation and survival signals [1,2]. Ample evidence has solidly implicated this tyrosine kinase receptor system in growth and survival of cancer cells [3]. Antibodies and tyrosine kinase inhibitors targeting the IGF-1R have been developed and their activity in multiple tumor types is now being tested in clinical trials [4].

A group of six IGF-binding proteins (IGFBPs) exist, that modulate IGF-1 actions, by regulating stability of IGF-1 in the circulation, transport of IGF-1 to target tissues and interaction with the receptors [5]. Besides the regulatory effects on IGF-1 functions, IGFBPs have been shown to exhibit IGF-1 independent actions, such as growth inhibition and apoptosis. Strong anti-apoptotic properties have been attributed to IGF-1s main binding protein IGFBP-3 [6].

IGF-1 plays a central role in integrating signals of nutrition and stress into energy shifts from energy-expensive anabolic processes, such as growth and reproduction, to preserving responses under catabolic or otherwise demanding circumstances, such as DNA damage [7,8]. Executing both metabolic and reproductive functions IGF-1 closely interacts with insulin and steroid hormones [4]. Also, apart from being genetically determined, IGF-1 and IGFBP-3 levels are believed to be influenced by anthropometric, reproductive, lifestyle, and dietary factors. Thus, IGF-1 is seen as a potential factor linking known influences of steroid hormones, body weight, fat metabolism, and insulin on cancer with each other.

High IGF-1 levels are by now a well-established risk factor in women for developing breast cancer [9,10]. A collaborative analysis of 17 prospective studies confirmed this finding and found that the association is not substantially modified by IGFBP-3, and seems to

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be confined to estrogen-receptor-positive tumors [11]. Biological ground for a causal relationship between IGF-1 levels and tumorigenesis comes from animal models. IGF-1 deficient mice show reduced growth and metastasis of tumors and xenografts and increased resistance to carcinogen-induced tumorigenesis [3]. Vice versa, transgenic overexpression of IGF-1 in basal epithelial cells induced well-differentiated adenocarcinomas [12,13]. The IGF-1R is overexpressed in many breast tumors and several IGF-1R inhibitors have recently entered clinical trials [4].

However, the effects of circulating IGF-1 and IGFBP-3 on disease recurrence and survival in breast cancer survivors are understudied. Four previous studies have investigated IGF-1 and prognosis in breast cancer patients, with varying patient numbers and study designs. These studies have shown inconsistent results. While associations of IGF-1 levels with decreased survival are found, these findings were not uniformly confirmed by others or are attributed to a confounding effect of tamoxifen use [14–16,21].

We hypothesized that high circulating levels of IGF-1 or low levels of IGFBP-3, through proliferative and anti-apoptotic effects, would be associated with an increased risk of second primary breast tumors and decreased overall survival in breast cancer patients. In a large cohort of breast cancer patients, with available plasma samples, clinical outcome parameters and reproductive history, we explored the effect of IGF-1 and IGFBP-3 levels on overall survival, occurrence of second primary breast tumors and environmental factors that could modify this risk.

Materials and methods

The study cohort consists of 1851 breast cancer patients, enrolled between 1998 and 2003 in a study designed to investigate low-penetrance genetic mutations predisposing to breast cancer [17,18]. Patients were accrued from six hospitals in the northern Netherlands (the University Medical Center in Groningen, and Medical Centers in Leeuwarden, Harlingen, Drachten, Dokkum and Sneek). In Drachten and Dokkum, breast cancer patients were identified through the regional cancer registry (Comprehensive Cancer Center Northern Netherlands). In the other hospitals, all breast cancer patients visiting the outpatient clinic for follow-up visits over a 1-year period were asked to participate by their physician. No selection was performed, only patients with a known BRCA1 or BRCA2 mutation were excluded.

Data on (self-reported) reproductive factors, height and weight, prior history of cancer, family history of cancer, smoking history and alcohol consumption were collected using questionnaires. Records on patient and tumor characteristics were updated and managed by the regional cancer registry. Follow-up was performed according to the regional follow-up guidelines (http://www.iknl.nl/page.php?nav_id=298&id=443) via the cancer registry. The mean follow-up period was 5.5 years (SD $\pm$ 2 years). Endpoints for the present study were overall survival and occurrence of second primary breast tumors. Overall survival is specified as death from any cause. No information on cause of death is available in the cancer registry dataset. Second primary breast tumors were defined as contralateral breast tumors, excluding local recurrences. The resulting time variables were calculated as time from diagnosis to the specified endpoints.

Indication for adjuvant treatment and selection of type of treatment was rule-based according to national treatment guidelines of the Dutch Association of Comprehensive Cancer Centers (http://www.oncoline.nl). Briefly, breast conserving surgery with axillary lymph node dissection was indicated for tumors $<$4 cm, complemented with radiotherapy to the breast. Alternatively, a modified radical mastectomy was performed. Loco-regional radiotherapy, consisting of parasternal, axillary, infra and supra-clavicular nodal irradiation, was indicated in case of $>$3 positive axillary nodes or extranodal growth. Pre-menopausal node-positive patients received adjuvant chemotherapy, generally consisting of cyclophosphamide, methotrexate and 5-fluorouracil. Anthracycline-based chemotherapy was increasingly used for high-risk patients since the mid-1990s. Adjuvant systemic therapy was also administered to node-negative patients with intermediate or poorly differentiated tumors larger than 1.1 cm. Furthermore, all hormone receptor positive, node-positive and unfavorable node-negative patients were to receive 5 years tamoxifen, irrespective of menopausal status. Inoperable and locally advanced cancers received chemotherapy, hormonal therapy and/or radiotherapy.

All samples and data in this study were anonymized and individuals were aware that they would not be informed about individual test results. All included patients gave written informed consent at enrollment in the study and 1566 out of 1851 patients had specified their consent for future studies. The Medical Ethical Committees of the participating hospitals approved the study.

Non-fasting 20-mL EDTA (ethylene diamine tetra-acetic acid) blood samples were collected at enrollment at the recruiting hospital or at the patient's home. Samples were delivered overnight by blood bank transportation running daily. On receipt at our laboratory, samples were kept in an air-conditioned laboratory at a temperature of approximately 20 °C, unopened and sheltered from direct sunlight until blood processing. Blood processing was done in clusters, delays until blood processing ranged from 2 to 5 days. Plasma samples were stored separately in small aliquots at −80 °C until time of analysis. Stability of IGF-1 and IGFBP-3 in EDTA plasma samples at room temperature with blood processing delays up to 7 days has been shown previously [19].

Owing to budgetary restrictions, total IGF-1 and IGFBP-3 were measured in 584 available plasma samples by automated chemiluminescence assay using Immulite system and reagents (Siemens Diagnostics, Los Angeles CA, USA). Two samples were lost for analysis due to a technical error. Samples had been collected within six years post-diagnosis (median 2 years). Intra- and interassay variability were estimated at two concentrations in at least 10 runs and two repetitions per run. Intra- and interassay variability of IGF-1 measurements were 1.7% and 2.1%, respectively, measured at 8.76 nmol/L, and 1.7% and 1.2% at 28.83 nmol/L. Intra- and interassay variability of IGFBP-3 measurements were 2.0% and 2.7%, respectively, measured at 0.94 μg/mL, and 2.7% and 1.0%, at 3.99 μg/mL. IGF-1 and IGFBP-3 concentrations showed a Gaussian distribution in our study population, overall and by hormonal treatment (Suppl. Fig. 1). The molar ratio IGF-1:IGFBP-3, used to estimate the biologically active fraction of IGF-1, was calculated based on the following conversion: 1 ng/ml IGF-1 equals 0.130 nM, 1 ng/ml IGFBP-3 equals 0.036 nM.

To exclude therapy-effects on IGF-1 and IGFBP-3 levels, IGF-1 and IGFPB-3 levels were measured prospectively in a separate cohort of breast cancer patients at our institution ($n = 29$) receiving adjuvant systemic chemotherapy and hormonal therapy [20]. Plasma samples were collected at diagnosis, one month after cessation of chemotherapy (FEC) and one month after start of endocrine therapy with Tamoxifen. No effect of chemotherapy on IGF-1 and IGFBP-3 levels was observed. IGF-1, but not IGFBP-3, levels decreased significantly during Tamoxifen treatment (data not shown), which is in line with previous reports [21–25]. Analyses including IGF-1 levels were subsequently performed separately in patient groups receiving endocrine therapy or not.

Data analysis was performed using the SPSS 16.0 statistical package (SPSS inc., Chicago, IL, USA). Associations of patient and tumor characteristics (age at diagnosis, menopausal status at diagnosis, tumor size, axillary lymph node status, distant